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Idarubicin-loaded ONCOZENE® drug-eluting bead chemoembolization in a rabbit liver tumor model: investigating safety, therapeutic efficacy and effects on tumor microenvironment

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Abstract

Purpose: To investigate toxicity, efficacy, and microenvironmental effects of idarubicin-loaded 40µm and 100µm drug-eluting embolic transarterial chemoembolization (DEE-TACE) in a rabbit liver tumor model.

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Conflict of Interest:

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Materials and Methods: 12 male New-Zealand White rabbits with orthotopically implanted VX2 liver tumors were assigned to DEE-TACE with 40 μ m (n=5) or 100 μ m (n=4) Oncozene® microspheres or as untreated controls (n=3). 24–72h post-procedurally, multi-parametric MRI (mpMRI) including dynamic contrast-enhanced (DCE), diffusion-weighted imaging (DWI), and biosensor imaging of redundant deviation in shifts (BIRDS) assessing extracellular pH (pH_e), was performed, followed by immediate sacrifice. Laboratory parameters and histopathological *ex-vivo* analysis included fluorescence confocal microscopy and immunohistochemistry.

Results: DCE-MRI demonstrated a similar degree of devascularization of embolized tumors for both microsphere sizes (mean arterial enhancement [%] 8 $\pm$ 12 vs. controls 36 $\pm$ 51, p=.07). Similarly, DWI showed post-procedural increases in diffusion across the entire lesion (apparent diffusion coefficient [x10–3mm²/s] 1.89 $\pm$ 0.18 vs. liver 2.34 $\pm$ 0.18, p=.002). BIRDS demonstrated profound tumor acidosis at baseline (mean pH_e tumor 6.79 $\pm$ 0.08 vs. liver 7.13 $\pm$ 0.08, p=.02), and after DEE-TACE (tumor 6.8 $\pm$ 0.06 vs. liver 7.1 $\pm$ 0.04, p=.007). Laboratory and *ex-vivo* analyses showed central tumor core penetration, and greater increase in liver enzymes for 40 μ m as compared to 100 μ m microspheres. Inhibition of cell proliferation, intra-tumoral hypoxia, and limited idarubicin elution were equally observed in both spheres.

Conclusions: Non-invasive MpMRI visualized chemoembolic effects on the tumor and tumor microenvironment following DEE-TACE. Devascularization, increased hypoxia, coagulative necrosis, tumor acidosis, and limited idarubicin elution suggest ischemia as the predominant therapeutic mechanism. Substantial size-dependent differences indicate a greater toxicity of the smaller microsphere diameter.

Introduction

Image-guided transcatheter intraarterial tumor therapies represent a fundamental pillar in cancer care of liver malignancies (1). Drug-eluting embolic chemoembolization (DEE-TACE) was originally developed to reduce systemic drug exposure seen with conventional oil-based TACE but failed to demonstrate any differences in outcome (2–5). Although various DEEs have been meanwhile developed, there is no standardized consensus regarding optimal embolic vectors, chemotherapeutic agents or optimal particle sizes (6–8).

In-vitro cytotoxicity data demonstrated that idarubicin was the most potent cytotoxic agent to HCC cells, which is likely due to its ability to overcome multidrug resistance (9). Loading, release, and dosing-safety of idarubicin in DEEs was successfully tested using the recently introduced Oncozene® DEEs (Boston Scientific, Marlborough, MA, USA) (10). They were shown to have a favorable pharmacokinetic profile and smaller calibratable DEEs (11, 12). Smaller-sized microspheres (<100 μ m) are, in fact, associated with more distal tumor vessel penetration, and thus superior drug distribution but were shown to pose contradictory features in terms of pharmacodynamics and clinical safety (6–8, 13). The effects of DEEs on the tumor microenvironment have not yet been investigated, though the tumor microenvironment plays a critical role in tumorigenesis (14). Among others, tumor response to hypoxia-induced changes of the tumor microenvironment including increased angiogenesis, tumor cellularity and extracellular pH, all of which are associated with therapy resistance, are poorly understood and could not be previously assessed in vivo (15–18). Understanding the effects of DEEs on these parameters may help gauge the role of various

anti-tumoral mechanisms in DEE-TACE and ultimately optimize clinical outcome. Therefore, the purpose of this study was to investigate safety, efficacy and microenvironmental effects of DEE-TACE with idarubicin-loaded 40µm and 100µm Oncozene® DEEs using non-invasive multi-parametric MRI (mpMRI) and *ex-vivo* histopathological and immunofluorescence analysis in a VX2 rabbit liver tumor model.

Materials and methods

Animal tumor model

Male adult New Zealand white rabbits (weight 4.3±0.2 kg, Charles Liver Laboratories) were used in accordance with institutional guidelines under approved Institutional Animal Care and Use Committee protocols as the appropriate tumor model between 02/2017 and 02/2018 (19, 20). Animals were maintained in laminar flow rooms at constant temperature and humidity, with food and water provided ad libitum. Tumor implantation was performed as previously described (21). In brief, VX2 tumors were injected into the hind legs of donor rabbits and grown for 14–21 days. Approximately 0.4 mL of the harvested tumors were implanted into the left hepatic lobe of recipient rabbits. The tumors were grown for 14 days until a delineated solitary tumor (1.5–2.0 cm in diameter) was measurable on CT (22).

Overall experimental design

Twelve rabbits were randomly assigned to DEE-TACE with 40µm (n=5) or 100µm (n=4) Oncozene® microspheres or as untreated controls (n=3). Pre-procedural CT was performed to evaluate tumor growth. Blood samples were obtained pre-procedurally, and at 5min, 24h and 48h post-TACE. Terminal mpMRI was acquired within 24–72 hours post-procedurally as for untreated controls to assess perfusion, diffusion, and extracellular pH (pH_e). Animals were sacrificed and necropsied immediately after image acquisition. Tumor and liver parenchyma samples were collected and processed for immunofluorescent and histopathological evaluation (Figure 1).

Transarterial chemoembolization procedure

TACE procedures were performed as previously reported (19, 23). Briefly, rabbits were initially sedated using ketamine (30–45 mg/kg body weight), acepromazine (2–3 mg/kg body weight), and xylazine (5–10 mg/kg body weight) and maintained with isoflurane (1.5–3%) and oxygen. A 3-french vascular sheath (Cook, Inc., Bloomington, IN, USA) was introduced into the femoral artery after blunt dissection. A 2-French microcatheter (JB1 catheter; Cook, Inc., Bloomington, IN, USA) was advanced into the celiac axis, where a celiac arteriogram was performed for tumor targeting. A 0.014" guidewire (Transend wire, Boston Scientific, USA) was selectively placed in the segmental tumor-feeding hepatic artery. The microsphere suspension was prepared with 15 mg idarubicin per 3 mL microspheres in either 40µm or 100µm diameter, added to 7.5 mL contrast (Omnipaque 350, GE Healthcare) and 7.5 mL distilled sterile water, and aliquoted into a 0.5mL syringe for delivery. Once the adequate position of the microcatheter was confirmed, the microsphere suspension was slowly infused under fluoroscopic guidance. Endpoints of embolization were complete administration of microsphere suspension or premature stagnation of blood flow, confirmed by intraprocedural fluoroscopy (24). Digital subtraction angiography,

fluoroscopy imaging and cone-beam CT were performed with a C-arm unit (Allura Clarity FD20, Philips Healthcare, Best, The Netherlands).

Blood laboratory analyses

Blood samples were obtained from the central ear artery or intra-procedurally through the femoral artery access prior to TACE, and at 5min, 24h, and 48h post-procedurally, based on the completion time of microsphere suspension (23). Laboratory analysis consisted of complete blood count, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, gamma-glutamyltranspeptidase, total bilirubin, albumin, and blood urea nitrogen. Vascular endothelial growth factor (VEGF) was determined at the same time points using the Rabbit VEGF ELISA kit (MyBioSource, Inc., San Diego, CA, USA).

Image acquisition and analysis

Contrast-enhanced multidetector CT was acquired pre-TACE (Figure 2). Within 24–72 hours after TACE, mpMRI was performed on a 3 Tesla scanner (Prisma Siemens, Erlangen, Germany) using a 15-channel RF coil and included T₁-weighted and T₂-weighted sequences for anatomical imaging, and dynamic contrast-enhanced (DCE)-MRI to evaluate blood perfusion. Diffusion-weighted imaging (DWI)-MRI was performed for diffusion and cellularity evaluation, and Biosensor Imaging of Redundant Deviation in Shifts (BIRDS) to quantify tissue acidity (24, 25). For detailed descriptions of MRI sequences and data processing please refer to the Supplementary material.

Histopathological Staining

Immediately after MRI acquisition, animals were sacrificed by intravenous injection of euthasol (0.5 mL/kg). During necropsy, one tumor slice was placed on optimal cutting temperature cassettes, shock frozen in liquid nitrogen and stored at –80°C. The remaining tumor was immediately placed in 10%-buffered formalin glass jars and stored at 4°C.

Representative formalin-fixed tumor and liver tissue samples harvested from the contralateral hepatic lobe were paraffin embedded in 5 mm slices. The tissue was then cut into 2-µm slices, deparaffinized using xylene, and rehydrated using a descending ethanol dilution series. After washing with deionized water, samples were permeabilized in boiling retrieval solution for 40 minutes at 95°C. All samples were stained with hematoxylin and eosin (H&E), proliferating cell nuclear antigen (PCNA) to display cell proliferation, and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) to visualize cell death (23). Tumor rims were quantified on H&E as an average of 5 measurements in different locations (26). Hypoxia marker pimonidazole (Hydroxyprobe, NPI Inc., Burlington, USA) was intraperitoneally injected (50 mg/kg) 2–4 hours before sacrifice as previously described (23). Tumor tissue samples were stained with pimonidazole antibodies (Hydroxyprobe, NPI Inc., Burlington, USA) accordingly, and hypoxia inducible factor-1 alpha (HIF-1α) to compare hypoxic extent.

Fluorescence Imaging

Representative formalin-fixed tumor samples were washed in deionized water and sucrose, embedded in optimal cutting temperature compound and cryosectioned in 10µm-thick slices

at -20°C using Leica cryostat (Leica Biosystems Imaging, Inc., Buffalo Grove, IL, USA). Most qualitative slides for imaging were stained with DAPI-PBS solution. Fluorescent images using blue 405 nm and green 488 nm wave-length channels were captured as single plane tiled images using an Olympus confocal micro-scope 4x objective. MetaMorph software image analysis was used to apply segmented histogram heat maps based on a previously established heat map scale for idarubicin drug signal analysis (Center for Biological Innovation, Boston Scientific, MA, USA) and performed using Metamorph microscopy automation and image analysis software (Molecular Devices, LLC., San Jose, CA, USA).

Statistical analysis

Since the end-points are semi-quantitative in nature, a size of $n=3$ was chosen as the balance between scientific necessity and resources available to perform the study in agreement with mandatory Animal Care and Use Committee limitations in USDA-restricted species, making it necessary to keep the numbers of animals used as low as reasonably achievable to meet study goals. Results of the experiments were summarized as mean $\pm$ standard deviation. Normality testing was performed for each group. Primarily, the Kruskal-Wallis test was used to compare rabbit characteristics and the Mann-Whitney U test to further explore differences among the groups. A two-tailed $P < .05$ was considered statistically significant. Statistical analysis was performed with R (R Project for Statistical Computing, Open Source, version 1.0.143/2017).

Results

Chemoembolization procedure

Pre-procedural tumor size measured on CT was 16.5 ± 3.4 mm. TACE was technically successful in all 9 animals including catheter advancement into the segmental hepatic artery and transarterial therapy administration. Post-procedural cone-beam CT revealed successful tumor embolization in all 9 animals by visualization of trapped contrast within tumor tissue (Figure 2). Flow stasis slightly prior to complete dose administration was achieved in 4 animals ($40\mu\text{m}$ $n=2$, $100\mu\text{m}$ $n=2$) with $>90\%$ of the dose being delivered. Administration of microsphere suspension was completed in 5 animals ($40\mu\text{m}$ $n=3$, $100\mu\text{m}$ $n=2$). The mean total volume of delivered microsphere suspension was slightly higher in $40\mu\text{m}$ as compared to $100\mu\text{m}$ microspheres (mean total volume [mL] 0.49 vs. 0.41 , $p = .04$). Complications in suspension delivery occurred in $100\mu\text{m}$ microspheres due to clumping within the microcatheter towards the end of the procedure ($n=2$). Other adverse events included femoral artery hemorrhage ($n=1$) and cardiac arrest under anesthesia during terminal imaging ($n=1$).

Laboratory analyses and safety profile

Animals tolerated idarubicin with no apparent drug-induced toxicity such as pain, pallor, immobility, anuria determined by daily clinical assessment, or weight loss. Blood analysis revealed no hematologic toxicity or abnormal renal function. In animals treated with $40\mu\text{m}$ DEEs, an 18-fold increase in ALT serum levels and a 25-fold increase in AST was observed within 24h post-procedurally compared to baseline (ALT 430.2 ± 165 vs. 23.6 ± 8.1 , $p < .001$).

and AST 434.2 ± 334 vs. 17.4 ± 5.9 , $p=.02$). Both, ALT and AST increases stagnated after 24h. 100 μ m DEEs caused slight increases in ALT (18 ± 5 vs. 86 ± 50 , $p=.07$) and no change in AST levels (29 ± 14 vs. 93 ± 64 , $p=.16$) (Figure 3). All other liver function parameters remained within reference. In both treatment groups, macroscopic hepatic necrosis was visible as dry tissue discoloration in the tumor segment and immediate vicinity. Additionally, 40 μ m DEEs also caused considerable hepatic necrosis in liver segments of the same lobe, not immediately adjacent to the tumor segment, indicating a non-target dispersion of 40 μ m microspheres. No extra-hepatic deposition of the embolics was observed.

VEGF plasma levels, indicative of angiogenesis, showed no differences in concentration among all groups ($p=.10$). VEGF expression remained constant after tumor embolization in all treatment groups (40 μ m 0.25 ± 0.1 , 100 μ m 0.28 ± 0.03 ng/ml, $p=.16$, Suppl. Figure 1).

Quantification of molecular imaging parameters

DCE-MR and DWI-MR images were obtained 2.5 $\pm$ 1.1 days post-procedurally from 11 rabbits (controls $n=3$, 40 μ m $n=5$, and 100 μ m $n=3$). BIRDS imaging could only be completed in 10 rabbits due to adverse events as detailed above (controls $n=3$, 40 μ m $n=4$, 100 μ m $n=3$). The average post-embolic tumor diameter was 16 ± 6 mm.

In controls, DCE-MRI showed a hyperenhancement of the tumor rim; the tumor core remained hypovascular (Figure 4). Equally in 40 μ m and 100 μ m DEEs, the tumor rim and tumor core were hypovascular (mean arterial enhancement [%] 40 μ m 2 ± 3 , 100 μ m 9 ± 3 , control 36 ± 51 , $p=.07$). Embolized tumors showed less enhancement in arterial and late portal venous phase compared with liver parenchyma (liver 81 ± 25 , $p<.001$, Figure 5).

In DWI of controls, lowest ADC values were obtained in the tumor rim (mean ADC $(1.55 \pm 0.09) \times 10^{-3} \text{ mm}^2/\text{s}$), without difference to liver parenchyma (1.62 ± 0.23 , $p=.65$). Corresponding tumor cores presented high ADC values (2.34 ± 0.18 , $p=.001$). After chemoembolization, tumor rims could not be distinguished and post-embolic lesions showed significantly higher ADC values than baseline tumors (40 μ m 1.91 ± 0.15 , 100 μ m 1.85 ± 0.27 , control 2.34 ± 0.18 , $p<.001$).

In untreated tumor tissue, BIRDS demonstrated lower pH_e values compared to liver parenchyma (mean tumor pH_e 6.79 ± 0.08 , liver 7.13 ± 0.08 , $p=.02$, Figure 4). Tumor rims defined as voxels partly (>50%) outside the tumor mask showed less acidity than tumor cores (7.03 ± 0.14 vs. 6.79 ± 0.08 , $p=.07$). Embolized tumors had similar results (tumor/liver pH_e 40 μ m $6.75 \pm 0.05/7.05 \pm 0.07$, 100 μ m $6.9 \pm 0.1/7.1 \pm 0.03$, $p=.003$, Figure 4), with no difference between treatment groups. Healthy liver parenchyma showed consistent pH_e values in all groups ($p=0.4$).

Ex-vivo histopathological evaluation

The multiparametric imaging findings were histopathologically confirmed by H&E, PCNA and TUNEL stainings. Untreated tumors showed scattered tumor core necrosis with viable tumor cell islands surrounded by a viable tumor rim (4 ± 0.6 mm) with densely packed tumor cells and high expression levels of proliferation marker PCNA (Figure 6). Hypoxic regions and cell apoptosis were strictly confined to the tumor core. In embolized tumors, no viable

tumor cells were identifiable, but sporadic cell outlines without nuclei highly suggestive of coagulative necrosis. This was confirmed by high expression levels of TUNEL and the disappearance of PCNA expression. TUNEL staining extended up to 25% of the tumor diameter into adjacent liver parenchyma in 40 μ m, but not in 100 μ m treatment group. The hypoxia markers HIF-1 α and pimonidazole corresponded to perishing regions of the tumor but exceeded tumor margins and were detectable in a radius of up to 50% of the tumor diameter in 40 μ m and up to 10% in 100 μ m microspheres (Figure 6).

Fluorescence imaging revealed that there is a greater quantity of intratumoral blood vessels containing 40 μ m embolics compared to 100 μ m microspheres (26.3 $\pm$ 9.1, 3.5 $\pm$ 2.1 blood vessels per tumor, $p=.04$). 40 μ m DEEs fully penetrated the tumor core, whereas 100 μ m embolics were mostly detected within the tumor rim area. Results also show that idarubicin was present within the spheres but merely eluted within a 100 μ m radius from embolized vessels in both TACE groups (Figure 7).

Discussion

The main finding of this study is that Oncozene® DEEs in both tested sizes induce anti-tumoral changes visualized on non-invasive mpMRI. The smaller-sized embolics showed penetration of more distal vasculature in the tumor and non-tumoral liver parenchyma, potentially leading to increased hepatic toxicity. However, both embolics caused rapid devascularization, broad hypoxia and consecutive coagulative necrosis with unaffected high acidity levels and limited idarubicin elution, suggesting ischemia as the predominant mechanism of action.

The chemo-embolic effects of this study confirmed previous observations, that smaller microspheres are associated with more distal tumor vessel penetration and consecutive tissue ischemia at the expense of higher tissue toxicity (6–8, 27). In this study, the 40 μ m DEEs dispersed across the tumor-bearing hepatic lobe, possibly through hepatic shunts or distal microcirculation (8, 28) inducing hypoxia in extratumoral liver parenchyma, which likely reflects injury of healthy tissue. Importantly, hypoxia is associated with increased neoangiogenesis and tumorigenic effects (6). In this study, however, no significant increase in VEGF plasma concentration was detected. This might be explained by the hypervascular nature of VX2 with already high baseline VEGF levels and the prolonged initiation of VEGF expression through gene transcription (29). Moreover, while post-embolization transaminitis has been previously reported as a transient phenomenon, reaching baseline levels after 5–7 days (19, 30), in this study, 40 μ m microspheres caused up to 5-fold higher levels of AST and ALT compared to 100 μ m microspheres, thus rendering 40 μ m DEEs toxicity potentially unbearable. Both sphere sizes showed similar anti-tumor potency with equally limited extents of idarubicin elution. While 40 μ m DEEs advanced into distal tumor vasculature, 100 μ m microspheres mainly remained marginal and therefore primarily released idarubicin in the outer tumor circumference. Despite the difference in idarubicin coverage, both embolics equally induced coagulative necrosis. According to Hong et al., the highest post-embolic drug concentration is measured 3 days post-treatment with thereafter continuous elution from the microspheres (19). It is reasonable to assume that no considerable increase in idarubicin coverage and its cytotoxic effect should be expected, had

the experiment included animal sacrifice at a further time point, since immediate, consistent tumor necrosis was already documented on imaging 48h post-procedurally (31). The findings suggest that tumor necrosis was achieved mainly independent of idarubicin effects and that ischemia due to devascularization is the predominant short-term therapeutic mechanism.

Multi-parametric MRI confirmed these DEE-TACE-induced effects. The DWI-derived ADC value is influenced not only by molecular diffusion, but by capillary perfusion (32) which further confirms anti-tumoral effects induced by devascularization. Furthermore, the MR spectroscopy-based quantification of pH_e using BIRDS addresses an unmet clinical need for non-invasive imaging parameters to detect metabolic abnormalities in TACE (33, 34). BIRDS is favorable for its independence of tissue perfusion, contrast agent concentrations and temperature variations (34). Tumor acidosis was detected with a gradient from very low pH_e levels within the tumor core towards higher levels within the tumor rim, the interpretation of which was slightly limited by resolution (34). The measured tumor acidosis might be explained by what is widely known as deranged tumor metabolism with almost exclusive reliance on aerobic glycolysis (Warburg effect) and rapidly progressive tumor growth (35). No significant post-embolic change in tumor pH_e was measured most likely due to the immediate onset of vascular occlusion with subsequent hypoxia which potentially exacerbates tumor glycolysis and subsequent acidification of the tumor microenvironment (36). Future experimental designs should apply longitudinal monitoring of post-TACE changes to evaluate the effects of devascularization and drug-deposition within the tumor rim on local pH_e and tumor progression.

This study has several limitations. Clinical and safety data of idarubicin-eluting microspheres are limited by both the small sample size and the short follow-up time. While longitudinal outcomes were not investigated as endpoints, survival studies with multiple pre- and post-procedural MRI and pharmacological drug analyses would provide a better evaluation of long-term efficacy, toxicity and tumor microenvironment transformation. Another model-related limitation is the inherent aggressiveness of the VX2 tumor model which frequently develops central necrosis at baseline. This inherent tumor tissue necrosis may impact the overall assessment of embolic effects to the tumor core which somewhat limits the relevance of histopathological and radiological findings. Recently established approaches in rodent models seem promising despite their dissimilarity to human vascular anatomy and inability to offer super-selective embolization technique (24, 25, 37).

In conclusion, this study showed that both 40 and 100 μm idarubicin-eluting Oncozene® microspheres demonstrated significant anti-tumoral effects, with ischemia rather than anticancer drug activity as the predominant mechanism of action. The apparent size-dependent differences in depth of vascular penetration, extra-tumoral deposition, extent of hypoxia and tissue toxicity suggest a higher risk and toxicity profile of the smaller embolics. The use of non-invasive multiparametric MRI and specifically the application of quantitative pH_e mapping reliably demonstrated therapy-related changes and may be used to identify HCC in earlier stages. Nevertheless, additional and longitudinal studies are warranted to establish those techniques as reliable early markers for TACE-induced effects on the tumor microenvironment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

DEE-TACE	drug-eluting embolic transarterial chemoembolization
mpMRI	multi-parametric magnetic resonance imaging
DCE	dynamic contrast-enhanced
DWI	diffusion-weighted imaging
ADC	apparent diffusion coefficient
BIRDS	biosensor imaging of redundant deviation in shifts
pH_e	extracellular pH
PCNA	proliferating cell nuclear antigen
TUNEL	terminal deoxynucleotidyl transferase dUTP nick end labeling
HIF-1α	hypoxia-inducible factor-1-alpha

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Figure 1: Experimental study design

12 rabbits were implanted in the left liver lobe with tumors harvested from donor rabbits. Tumors were grown for 14 days until multidetector computed tomography (MD-CT), transarterial chemoembolization (TACE) and cone-beam computed tomography (CBCT) were performed. Blood samples were obtained at specific time points. Multi-parametric MRI (mpMRI) was performed 24–72 h post-procedurally, after which animals were immediately euthanized and necropsied for radiological-histopathologic evaluation.

Figure 2: Pre- and post-procedural CT imaging

White arrows indicate the tumor in pre- and post-procedural contrast and non-contrast CT imaging in both treatment groups. Post-TACE cone-beam non-contrast CT demarcates successful tumor embolization by visualization of trapped contrast medium within tumor tissue.

Figure 3: Laboratory analyses of liver transaminases

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) serum levels (U/l) pre-procedural and 5 min, 24 h and 48 h after chemoembolization in both treatment groups (40 μ m blue and 100 μ m red) and controls (black). A higher increase of both ALT and AST was observed in 40 μ m DEEs compared to 100 μ m DEEs.

Figure 4: Multiparametric MRI sequences in representative control and embolized animals
 White arrows indicate the tumor. T1-weighted, non-contrast MRI in axial plane indicate tumor location. Contrast-enhanced T1-weighted images in arterial phase show a tumor rim enhancement in controls and which disappears in embolized animals. Dynamic contrast-enhanced MRI (DCE-MRI) demarcates the tumor rim as a hyperenhanced outline that becomes undistinguishable in embolized animals suggestive of tumor devascularization. The apparent diffusion coefficient (ADC) map demarcates an increase in tumor diffusion in embolized animals. The last row represents pH_e mapping with BIRDS. The tumor (red outline) is more acidotic (blue voxel color) than corresponding liver parenchyma (blue outline).

Figure 5: Time-enhancement-curves on dynamic contrast-enhanced MRI

The tumor is depicted in black, arterial enhancement in red and liver enhancement suggestive for the portal venous phase is visible in green. Overall, arterial and liver enhancement are subject to individual fluctuations but do not differ in all three animals. In controls, the tumor shows a strong enhancement whereas in both treatment groups the tumor remains completely unenhanced suggesting a successful embolization and thus devascularization of the tumor.

Figure 6: Histopathological correlation of imaging findings

Histology included H&E, PCNA, TUNEL, HIF-1α and pimonidazole staining (vertical rows) in treatment groups (horizontal rows). Control tumors demonstrated a viable, proliferating tumor rim with high expression levels of PCNA and fragments of necrosis and corresponding hypoxic regions confined to the tumor core. In embolized tumors, no viable tumor cells were identifiable confirmed by a disappearance of PCNA and upregulation of TUNEL expression. Expressions of hypoxic markers HIF-1α and pimonidazole exceeded tumor margins and were detectable in a radius of >50% of the tumor diameter in 40μm and >10% in 100μm microspheres.

Figure 7: Immunofluorescence and histological correlation

Immunofluorescence (IF) and H&E in a representative animal of 40μm and 100μm. A, D: H&E staining of embolized vessels containing idarubicin-loaded spheres. B, E: IF staining of tumor (blue outline) with background DAPI staining and idarubicin auto-fluorescence (green). Idarubicin drug signal is detectable peri-vascular in both DEEs. C, F: IF staining with segmented histogram heat map for quantification of idarubicin drug concentration.